Inactivation of MS2 coliphage in sewage by solar photocatalysis using metal-doped TiO$_2$

Danae Venieri [a,*], Iosifina Gounaki [a], Vassilios Binas [b,c], Apostolos Zachopoulos [b], George Kiriakidis [b,c], Dionissios Mantzavinos [d]

[a] *School of Environmental Engineering, Technical University of Crete, GR-73100 Chania, Greece*
[b] *Institute of Electronic Structure and Laser (IESL), FORTH, Vasilika Vouton, GR-70013 Heraklion, Greece*
[c] *Quantum Complexity & Nanotechnology Center (QCN), Department of Physics, University of Crete, GR-70013 Heraklion, Greece*
[d] *Department of Chemical Engineering, University of Patras, Caratheodory 1, University Campus, GR-26504 Patras, Greece*

A B S T R A C T

The beneficial properties of metal-doped titania nanoparticles with respect to wastewater disinfection under solar irradiation were investigated. Mn-, Co- and binary Mn/Co-doped TiO$_2$ catalysts were prepared by means of a co-precipitation method and were subsequently assessed in terms of their potential to inactivate MS2 bacteriophage in slurry reactor under simulated and natural solar irradiation. Disinfection effectiveness was evaluated in relation to influential operating parameters, like catalyst type (Mn-, Co- and Mn/Co), dopant concentration (0.02–1 mol wt%), artificial and natural solar light, wavelength (i.e. >420 nm) and photon flux (4.93–5.8 × 10^{-7} E/(Ls)). Metal doping led to considerable narrowing of the band gap and the spectral response of the catalysts extended well into the visible region. MS2 phage was readily inactivated in sewage samples under simulated solar irradiation in the presence of the prepared metal-doped catalysts. The latter proved to be superior to the commercial P25, under the current experimental conditions, resulting in an approximately 60% phage population decrease in almost 60 min of simulated solar irradiation when initial MS2 concentration was 10^5 PFU/mL. Catalysts with the binary dopant exhibited the best photocatalytic activity in all cases, as almost 99% of MS2 population was eliminated in less than 20 min of irradiation highlighting the fact that composite dopants induce a synergistic effect. The effect of different dopants concentration was apparent up to a certain limit. Disinfection follows a pseudo-first order kinetic rate. Retardation of the process by a factor 1.6–3.8 was recorded under natural solar light, based on the kinetic rate constants of inactivation curves which were within the range of 0.032–0.057 min^{-1}. The corresponding range for inactivation under simulated solar irradiation was 0.053–0.221 min^{-1}. Moreover, testing the Mn-, Co-, and binary Mn/Co doped TiO$_2$ in the absence of UV light, they were considerably sensitized making clear that they can be activated in the visible part of the spectrum.

1. Introduction

The constant presence of bacteriophages in sewage and polluted waters has led to their use as appropriate indicators of fecal pollution, considering that their presence significantly denotes the occurrence of relative bacterial populations, which are capable of supporting their replication [1]. Moreover, these organisms are suggested as indicators of viral presence in the aquatic environment, as their basic features, namely, their size, morphology, structure and their overall behavior closely resemble those of enteric viruses

[2,3]. F-specific coliphages (male-specific phages), which infect *Escherichia coli* strains and related bacteria through the sex pili they possess, have received special attention due to their similarity to many pathogenic human enteric viruses [4,5]. For these reasons and due to their high numbers in wastewaters and their relatively high resistance in chlorination, coliphages are often used as an index of wastewater contamination and for evaluation of disinfectants [1,6].

In general, bacteriophages, like viruses, are characterized by their resistance to stressed environmental conditions imposed by sunlight, temperature fluctuations and to chemical and physical treatments. Therefore, they are considered possible indicators of treatment processes efficiency [2,7]. Commonly wastewater disinfection is achieved by chlorination, which is capable of inactivating a quite extended variety of microorganisms through cellular

destruction, impairment of enzyme and protein functions and nucleic acid denaturation [8–10]. However, in recent years, regulations mandate more stringent control of certain pathogens which exhibit high resistance to chlorine like parasites and viruses and of disinfection toxic by-products which may be toxic and genotoxic in the case of chlorination [11–13]. Although this technique remains the dominant disinfection process, other methods have emerged as quite effective in terms of water and wastewater disinfection.

Titanium dioxide (TiO$_2$) photocatalysis seems to be an attractive disinfection method, as it has already demonstrated high reduction of viral and bacterial infection ratios in water/wastewater samples [14–18]. Advantages of photocatalysis include the absence of residues, simultaneous treatment of diverse pollutant mixtures, broad range and ease of operation [19,20]. The wide band gaps of pure titania (3.2 eV for anatase and 3.0 eV for rutile) make this catalyst mainly active under only ultraviolet (UV) light, leading to the generation of reactive oxygen species (ROS), which finally cause oxidative stress to microbial populations. Physical and chemical interactions between these compounds and the organisms cause the inactivation of the latter in aquatic matrices [14–17,21].

However, the fact that TiO$_2$ can be excited under UV light, which only accounts for less than 5% of solar irradiance, limits its practical applications in many cases as, inevitably, solar source of energy is excluded. In this sense, many efforts have been made to narrow the band gap of TiO$_2$ phases and therefore to broaden the absorption spectrum of this semiconductor toward the visible light region [22–24]. Photocatalytic activity may be enhanced by doping TiO$_2$ with non-metals or/and noble and transition metals, which significantly influence the photoreactivity, charge carrier recombination rates, and interfacial electron-transfer rates [25]. These composite catalysts have been shown to have superior antiviral and antibacterial behavior compared to pure titania, due to their high surface area, the red-shift in their absorption spectrum to the visible range, while sustaining the generation of a sufficient number of excited electrons in the conduction band [15,22,26–29].

Photocatalysis with doped TiO$_2$ has been shown to be an effective disinfection technique for the removal of bacteria and viruses mostly from water samples with *E. coli* being the regular testing indicator [26,27,29]. Nonetheless, microbial inactivation profiles are altered significantly after treatment of samples with high complexity, like those of wastewater. Particulate matter present in wastewater and the respective turbidity aid in the resistance of microorganisms to disinfection, as there is a physical shield protecting organisms from any irradiation applied and its detrimental effect [1].

In this perspective, the objectives of the current study comprise the testing of manganese- (Mn-), cobalt- (Co-), and binary (Mn/Co-)-doped titania materials, which were prepared in previous work [30], in terms of MS2 coliphages inactivation in real wastewater samples under simulated solar irradiation. Disinfection effectiveness was evaluated in relation to influential operating parameters, like catalyst type, dopant concentration, as well as photon flux. Additional experiments were performed under natural sunlight. Furthermore, transmission electron microscopy (TEM) was employed for the visualization of intact and inactivated phages aggregates in experimental samples.

2. Experimental

2.1. Materials

All chemicals which were used for the preparation of metal-doped catalysts were purchased from Aldrich and were of analytical reagent grade with no further purification. The reagents applied in the study were titanium(IV) oxysulfate hydrate (TiOSO$_4$·xH$_2$O),

manganese(II) acetate tetrahydrate (Mn(CH$_3$COO)$_2$), cobalt(II) acetate tetrahydrate (Co(CH$_3$COO)$_2$) and ammonium hydroxide (25% NH$_4$OH). Commercially available titanium dioxide (TiO$_2$ P25) powder was purchased from Degussa-Evonik Corp. Its physico-chemical characteristics are anatase:rutile 75:25, primary particle size of 21 nm and its BET area is 50 m^2/g.

2.2. Preparation of metal-doped TiO$_2$

Metal-doped TiO$_2$ nanoparticles were prepared by a co-precipitation method with molar ratio in different concentrations in the range of 0.02 to 1 wt%. Details regarding synthesis and characterization of all catalysts used in the present study can be found in previous studies [25,30]. The crystal structure, particle size, and morphology were examined with powder X-ray diffraction (XRD), SEM, and TEM, respectfully. Powder X-ray diffraction patterns were collected on a Rigaku D/MAX-2000H rotating anode diffractometer (Cu$K\alpha$ radiation) equipped with the secondary pyrolytic graphite monochromator operated at 40 kV and 80 mA over the 2θ collection range of 10–80°. The scan rate was 0.05° s^{-1}. The UV–visible diffuse reflectance spectra of the final powders were measured on a Perkin Elmer LAMBDA 950 with BaSO$_4$, as reference standard. The UV–visible diffuse reflectance spectra of the final powders were measured on a Perkin Elmer LAMBDA 950 with BaSO$_4$, as reference standard. The diffuse reflectance spectra were plotted as the Kubelka–Munk function, $F(R)$, versus wavelength based on the Kubelka–Munk equation: $F(R) = (1 - R)^2/(2R)$, where the reflectance is $R = R_{sample}/R_{reference}$. Band gaps were then determined from the Kubelka–Munk function and the Tauc plots. Surface morphology and elemental analysis of the samples were carried out using scanning electron microscopy (SEM) and an energy dispersive spectrometer (EDS) on a JSM-6390LV instrument. MS2 phages were studied by transmission electron microscopy (TEM) working at 80 kV (JEM-2100 instrument equipped with LaB6 filament).

2.3. MS2 coliphages culture and analysis

MS2 coliphages (ATCC 15597-B1) and its bacterial host *E. coli* (ATCC 15597) were obtained from the American Type Culture Collection (Manassas, VA 20108 USA). Bacteriophages were propagated and quantified by the plaque assay method (soft agar double layer), which is validated by ISO 10705-1. The host was grown in tryptone–yeast extract-glucose broth (TYGB) and was used in the exponential to early stationary phase. Bacteriophage stocks were produced by inoculating an exponentially growing culture of the host with MS2. After 4–5 h of incubation chloroform was added to lyse bacterial cells and the mixture was centrifuged ($3000 \pm 200 \times g$ for 20 ± 5 min) to remove cell debris. The MS2 culture was then titrated using *E. coli* host strain and inoculated tryptone–yeast extract-glucose agar (TYGA) plates were incubated at 37 °C overnight. Virus concentrations are reported as plaque forming units (PFU) per milliliter (mL) of sample assayed in each experiment.

2.4. Disinfection experiments

Disinfection experiments were carried out with real wastewater samples collected from the municipal wastewater treatment plant (117,500 equivalent inhabitants) located in Chania, W. Crete, Greece. Sampling took place from the effluent of the biological treatment process (activated sludge) sludge unit prior to disinfection (chlorination). Average values of wastewater chemical parameters were as follows: the chemical oxygen demand and dissolved organic carbon were 26 and 7.8 mg/L, respectively, the concentration of chlorides, sulfates, nitrates, nitrites, bicarbonates and total solids were 222.1, 60.3, 25.9, 57.1, 182.1 and 7 mg/L,

respectively, while the pH was 7.8. The period of sampling was from March until the end of May of 2014. All samples were auto-claved prior to photocatalytic treatment and were inoculated with the desired titer of MS2 bacteriophage stock cultures.

Photocatalytic experiments were conducted in batch type, lab-oratory scale photoreactor. Solar irradiation experiments were carried out in a solar radiation simulator system (Newport, model 96000) equipped with a 150 W xenon ozone-free lamp and an Air Mass 1.5 Global Filter (Newport, model 81094), simulating solar radiation reaching the surface of the earth at a zenith angle of 48.2°. The spectral output of the lamp is from 200 nm to approx-imately 2400 nm. According to the spectral irradiance data given by the manufacturer, simulated solar radiation contains about 5% UV-A radiation, and 0.1% UV-B radiation, while the filter cuts radia-tions with wavelengths lower than 280 nm. The incident radiation intensity on the photochemical reactor in the UV region of the electromagnetic spectrum was measured actinometrically using 2-nitrobenzaldehyde (Sigma-Aldrich) as the chemical actinometer [31,32] and it was found to be 5.8×10^{-7} E/(L s), which corre-sponds to an irradiance of $7.5\,W_{UV}/m^2$. Actinometric measurement was performed under the same conditions as those of disinfec-tion experiments. Additional experiments were performed with filters purchased from NEWPORT as follows: (i) with a 420 nm cutoff filter to remove all the UV light (FSQ-GG420 colored glass fil-ter, 50.8 mm × 50.8 mm, cut-on colored glass filter made of GG.420 glass), (ii) with a filter (FSQ-ND02, 50.8 mm × 50.8 mm, 80% trans-mittance at 632.8 nm) to reduce irradiance to 5.3×10^{-7} E/(L s), and (iii) with a filter (FSQ-ND04, 50.8 mm × 50.8 mm, 40% trans-mittance at 632.8 nm) to reduce irradiance to 4.93×10^{-7} E/(L s). FSQ-ND02 and FSQ-ND04 Fused Silica metallic neutral density fil-ters are UV fused silica metallic ND filters with an optical density of 0.2 and 0.4 at 632.8 nm, respectively. Their metallic neutral density coatings provide broadband attenuation for lower power applica-tions. The coating is deposited on one side of an ultra-pure UV grade fused silica substrate providing excellent transmission from the UV to near-infrared.

Filters were used in order to further support and verify the visible-light activity of the prepared catalysts, as well as to highlight the contribution of UV light to the overall photocatalytic activity. Reactions took place in an open, double-walled, cylindrical glass vessel under continuous stirring.

In a typical run, the wastewater sample of 300 mL, which was phage titrated with 10^5 PFU/mL of MS2, was loaded in the reaction vessel with the appropriate amount of catalyst. Catalysts loadings were tested in the range of 25–250 mg/L. The solution was left in the dark under continuous stirring for 30 min in order to equilibrate and then exposed to solar irradiation; this moment was taken as the starting point (time zero) of the disinfection experiment. Temper-ature was maintained at 25 ± 2 °C with a temperature control unit. The external reaction vessel was covered with aluminum foil to reflect irradiation exerting the outer wall of the reaction vessel. At specific time intervals 1 mL of the reaction solution was withdrawn and analyzed with respect to MS2 bacteriophage PFU applying the soft agar double layer method, as already mentioned. Briefly, 1 mL of the reaction solution was diluted 1:1000 with 0.1 wt% peptone solution by making 10-fold dilution series. Then, 1 mL of each dilu-tion was mixed with 1 mL of a Log phase broth culture of the host strain (E. coli ATCC 15597) and 3 mL of melted 1% TYGA and after gentle homogenization they were poured over petri dishes contain-ing bottom agar (2% TYGA). The plates were gently swirled, dried for 10 min at room temperature and then inverted and incubated at 37 °C for 24 h. The number of plaques was counted on those plates that had between 30 and 300 plaques. All disinfection experiments were performed in triplicate.

Photocatalytic experiments were also carried out under natural sunlight within the period of June 2014 at the Technical University

of Crete campus located at 38°31′N and 24°04′E, in clear sunny days. During those runs the recorded ranges of solar irradiance and temperature were 12.7–13.4 W/m² and 29–32.7 °C, respec-tively. Each phages titrated wastewater sample was introduced into borosilicate glass bottle, which was magnetically stirred during all experiment. The total volume was 300 mL. The photocatalyst was added to the sample and after 30 min of stirring in the dark for homogenization it was exposed to natural solar irradiation.

TEM observation of phages was carried out before and after treatment with representative doped catalysts.

3. Results and discussion

3.1. Mn- and Co-doped TiO₂ nanoparticles

In the present study disinfection potential of Mn- and Co-doped TiO₂ nanoparticles was investigated. Detailed description of opti-cal and structural properties of metal-doped TiO₂ nanoparticles was presented in previous work [30]. Dopant concentrations were within the range of 0.02 to 1 wt% for Mn- and Co-doped and 0.04 to 0.1 wt% for the binary Mn/Co co-doped, respectfully. According to XRD analysis, the powders showed crystalline nature with crystals oriented in 101 plane of the anatase phase. Catalysts with 1 wt% of Mn- and Co- dopants, respectively, exhibited a mixture of phases with both anatase and rutile, whereas all others were monophasic with only the anatase polymorph TiO₂ being detected. UV–visible analysis of the powders, showed that the absorption spectrum of the doped catalysts shifted toward the visible range (400–800 nm), while the onset of absorption spectrum of P25 was found to be at ~387 nm, which corresponds well with the known band gap of the material (3.2 eV) [30]. The recorded absorption shifts of doped cat-alysts represent a decrease in the band gap and subsequent visible light absorption.

Diffuse reflectance spectroscopy was used to investigate the light absorption capability and the band gap energy of the pho-tocatalysts. The band gap was determined from the Kubelka–Munk plots, the plot of $[F(R) \times h\nu]^{1/2}$ $(eV)^{1/2}$ versus photon energy (Fig. 1). Band gap energy of all prepared catalysts was below 3.2 eV, energy which is necessary for the excitation of P25. As it may be observed in Fig. 1, an increase of the dopant concentration resulted in a sig-nificant decrease in the band gap energy. Photocatalysts with the lowest dopant concentration (0.02 wt%) of Mn- and Co- displayed values of 2.7 and 2.97 eV, respectively, while the highest dopant concentration (1 wt%) led to further decrease and the band gap was found to be 2.4 eV. Additionally, Mn/Co co-doped catalysts of 0.04 and 0.1 wt% dopant concentrations had band gap energy of 3 and 2.7 eV, respectively. The lower band gap energy in Mn-, Co- and binary Mn/Co doped catalysts were expected to play key role in their response to visible light and the overall enhancement of their photoactivation in relation to wastewater disinfection [28].

Particle size of catalysts powders was in the range of 31.1–40.6 nm and 37.5–44.3 nm for Mn- and Co- doped titania and the respective size of binary doped TiO₂ did not exceed the value of 40.1 nm. According to SEM and TEM images there were no par-ticular changes in the morphology pattern of synthesized spherical particles in comparison with pure titania. What was demonstrated in the figures was a trend for surface particle agglomeration, which may in turn decrease the surface area of the nanoparticles by pro-ducing closed pores. Diameter of spherical particles ranged from 0.1 to 40 μm.

3.2. Doped titania-mediated wastewater disinfection

Preliminary optimization experiments were performed with various catalysts concentrations, in order to obtain satisfactory MS2
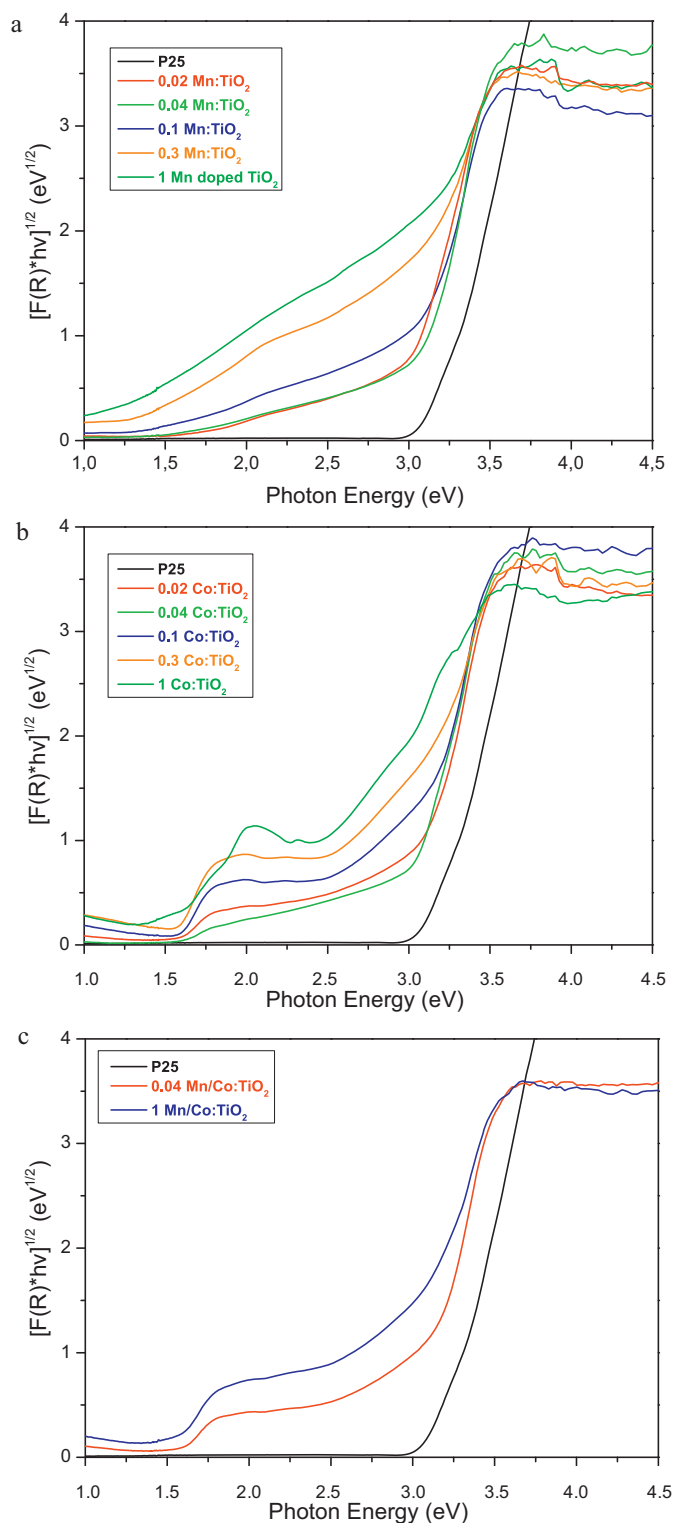
Fig. 1. Kubelka–Munk plot of (a) Mn-doped TiO_2, (b) Co-doped TiO_2 and (c) binary Mn/Co doped TiO_2 catalysts. Data for the commercial P25 sample are also shown.

concentration beyond 100 mg/L did not enhance substantially the overall process. With the view not to further increase the already high turbidity of our samples we chose the loading of 100 mg/L for disinfection experiments. Higher TiO_2 concentrations would dramatically reduce light transmittance into the bulk of the suspension, making catalysts activity less effective, as there would be a lack of adsorption of the viruses onto TiO_2 nanoparticles [11,12]. Similar catalyst concentrations have been employed in other photocatalytic decontamination studies, which mostly refer to diverse microbial elimination namely, *E. coli*, *Klebsiella* sp., *Pseudomonas* sp. *Enterococcus* sp., MS2 bacteriophage and others in aquatic matrices [13–18,33,34]. Generally, optimal catalyst loadings vary among studies as they depend on many operating parameters, with the type of microorganism affecting efficiency to a considerable extent.

Moreover, the possible biocidal nature of the metals, which were used for the preparation of the catalysts, was assessed with experimental runs performed in the dark. Catalysts with the lowest (0.02 wt%) and highest (1 wt%) concentration of metal dopants were loaded in wastewater samples, which contained 10^5 PFU/mL of MS2 phage and left in the dark for almost 1 h. Phage population remained stable within this period, showing that the metal-doped catalysts were not toxic to MS2 regarding their short-term toxic effects.

3.2.1. Effect of catalyst type

Prepared doped catalysts were tested in terms of their potential to eliminate MS2 phages in wastewater samples. All catalysts showed phages inactivation ranging from 1 to 5 Log population reduction within 60 min of treatment, as may be observed from Figs. 2 and 3. Dopants in all cases added to the photocatalytic activity of TiO_2 under the current experimental conditions, since there was an improved phages population reduction compared to the respective achieved with pure titania. The latter resulted in an approximately 60% phage population decrease in almost 60 min of simulated solar irradiation, while the vast majority of metal-doped nanoparticles led to over 99% inactivation of the tested bacteriophage in the same period of time. Moreover, it was observed that increasing the dopant concentration, disinfection efficiency was improved. In the case of Co- an increase beyond 0.3 wt% did not show any significant enhancement of the process. Both catalysts of 0.3 and 1 wt% led to a 99.9% reduction of phages within 30 min of treatment, whereas the same elimination rate was obtained only with Mn 1 wt%. However, when Co- doped titania was applied (e.g. 0.04 or 0.1 wt%), residual phages were observed in the bulk solution and enumerated plaques reached 4–20% of initial phage population after 60 min of treatment. This finding may be attributed to the specific properties of Co, which, as a transition metal, may act as a recombination site for the photo-induced charge carriers thus, lowering the quantum efficiency and its disinfection potential [20]. Significantly lower inactivation behavior of MS2 was showed in studies, in which undoped TiO_2 were used [16]. However, it should be noted the resistant nature of this phage in stressed environments, like those induced by advanced oxidation processes, in comparison with the more sensitive bacterial species found in water and wastewater [3]. The lack of enzymes, cellular structure and other sensitive systems makes viruses highly resistant over hydroxyl radical attack. Differences in size and surface properties between phages and bacteria cause diverse behaviors, with the former being more persistent during various disinfection methods in aquatic environments [1,3,7,16]. The only structure available for attack is the protein of the capsid they possess, which is simple and rigid, requiring more oxidizing power.

The overall phages inactivation in our samples was further improved when binary dopants were used in the photocatalytic process, with which 99.9% reduction of MS2 concentration was achieved in less than 20 min of irradiation. This finding highlights
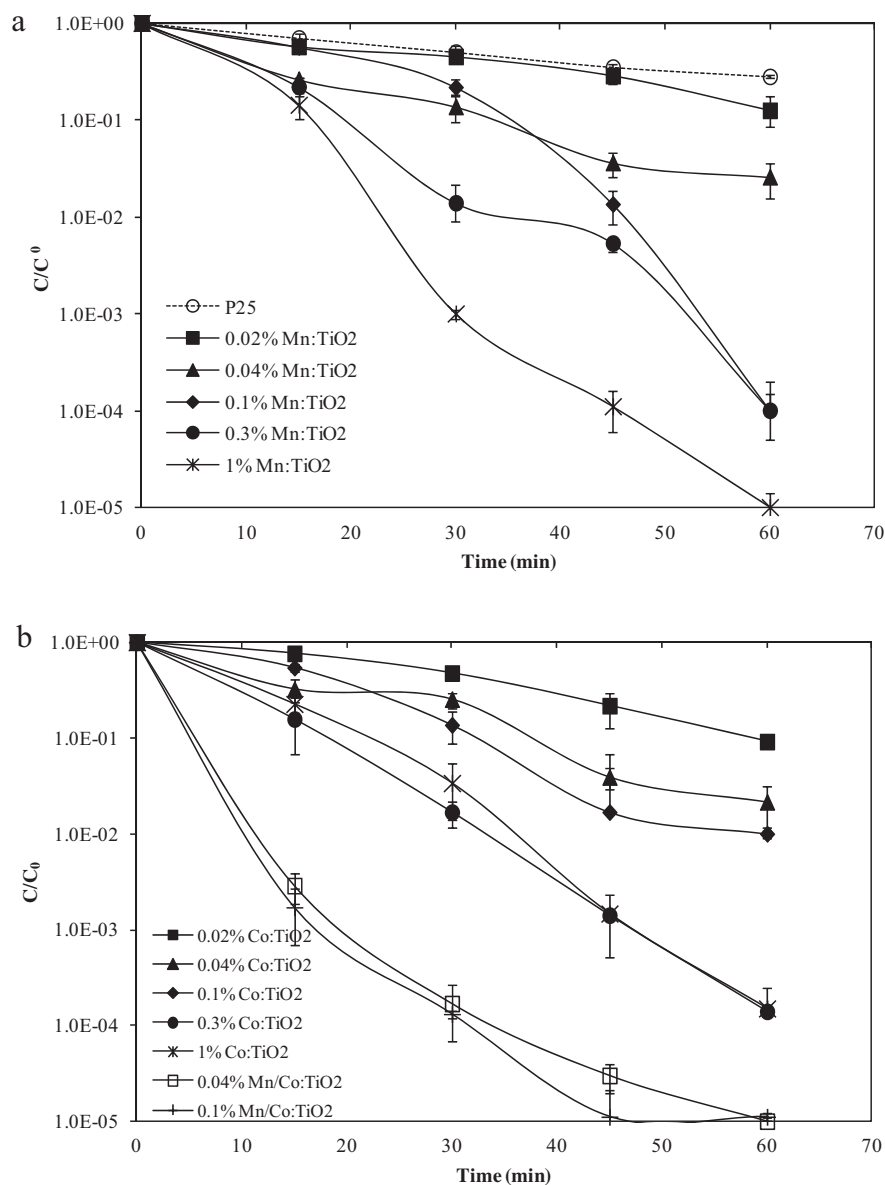
inactivation rates in wastewater samples. Catalysts loadings were tested with initial phage population of 10^5 PFU/mL in the range of 25–250 mg/L and led to variable decay rates, depending on the catalyst components and its concentration in each case. The loading of 100 mg/L resulted in satisfactory inactivation rates, i.e. an almost 5 Log reduction of phage population after 1 h of exposure under simulated solar radiation. Moreover, an increase of the catalysts

Fig. 2. MS2 phage inactivation in sewage samples under simulated solar irradiation and in the presence of different catalysts: (a) experiments with the commercial P25 and Mn-doped TiO$_2$ catalysts and (b) experiments with Co-doped and binary Mn/Co doped TiO$_2$ catalysts. Catalyst concentration is 100 mg/L and MS2 initial concentration is 10^5 PFU/mL.

the fact that composite dopants induce a synergistic effect overcoming the disadvantages of the individual components [8,20]. In the present study co-doped catalysts proved to have an optimal ratio of doping levels, which seems to define the activity of TiO$_2$. Fisher et al., who studied disinfection efficiency of 1% Cu/3.5% N and 1% Cu-doped catalysts, found that the former ones did not enhance bacterial inactivation, assuming that N atoms in the lattice may had acted as recombination centers for photogenerated electrons and holes [26]. This is consistent with other studies, indicating that catalytic behavior is strongly materials-dependent [35]. Certain limitations concerning dopants incorporation on TiO$_2$ surface have already been reported, of which photo-induced corrosion and promoted charge recombination at some metal sites seem to be the most significant [36].

In order to further investigate phages inactivation TEM analysis was employed and selected images are presented in Fig. 4. MS2 is visualized in the bulk reaction solution and in contact, possibly, with the catalysts nanoparticles, since all other debris was

removed during the preparation process of samples for TEM analysis. The inactivation behavior of MS2 phage is supposed to be mediated by free hydroxyl radicals in the bulk phase and not by those bound on the catalyst surface [16]. This hypothesis is based on the electrostatic repulsion between the TiO$_2$ particles and the MS2 phage, whose surface has both hydrophobic and negatively charged regions. According to Cho et al. and other studies the adsorption of the phage onto the surface of the particles is not favored and their contact should be minimal. Therefore, the overall inactivation process takes place in the bulk solution of the sample [37]. Although aquatic matrix would force MS2 particles into contact with TiO$_2$ particles, due to their hydrophobic regions, this would be situated at parts of the catalyst particle probably unsuitable for direct contact with hydroxyl radicals [38]. Conversely, Ditta et al. suggest that most phages are killed when bound to TiO$_2$ particles [21]. Phages may adhere to catalytic surfaces magnifying the effects of short-lived radical species [26]. However, the use of different suspension media should be of consideration, as their chemical synthesis plays
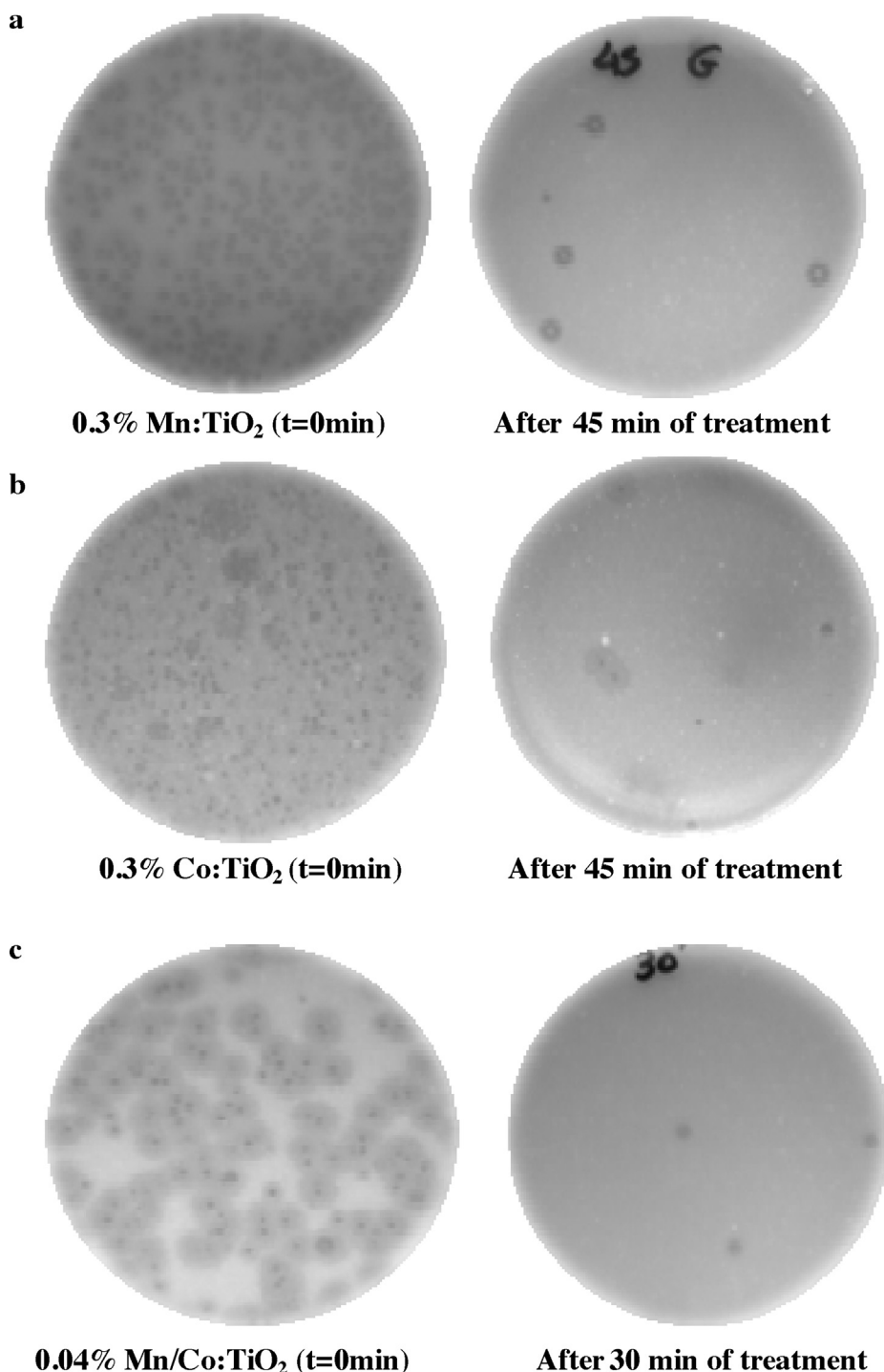
a

0.3% Mn:TiO$_2$ (t=0min)   After 45 min of treatment

b

0.3% Co:TiO$_2$ (t=0min)   After 45 min of treatment

c

0.04% Mn/Co:TiO$_2$ (t=0min)   After 30 min of treatment

Fig. 3. Plaques formation of MS2 in sewage samples after treatment with (a) Mn-doped, (b) Co-doped and (c) binary Mn/Co doped TiO$_2$.

significant role on the inactivation rate and the contact between the phage and the catalyst surface, which should not be prohibited when rapid disinfection is desired.

3.2.2. Natural sunlight-mediated phages inactivation

Additional disinfection experiments were performed in natural sunlight in order to compare inactivation results with those obtained with simulated solar irradiation. Under perfectly sunny conditions titrated wastewater samples were exposed outdoors in sunlight for 1 h and results are shown in Fig. 5. The catalysts selected for this set of experiments were some of those that achieved a phage inactivation more than 4 orders of magnitude in the

reaction solution (Fig. 2). During sunlight experiments ranges of solar irradiance and temperature were 12.7–13.4 W/m^2 and 29–32.7 °C, respectively, with average values of 13.1 W/m^2 and 30.1 °C. Also, no inactivation was recorded (a) in dark conditions with or without the catalysts in question and (b) under natural sunlight without the use of any catalyst. F-RNA phages are considered sunlight resistant organisms, which often exhibit higher tolerance in aquatic environments than other microbial indicators like fecal coliforms and enterococci [39]. Comparing rates between simulated (Fig. 2) and natural solar radiation (Fig. 5), it may be observed that they follow the same trend. Phages were rapidly inactivated in natural sunlight, however longer treatment periods were
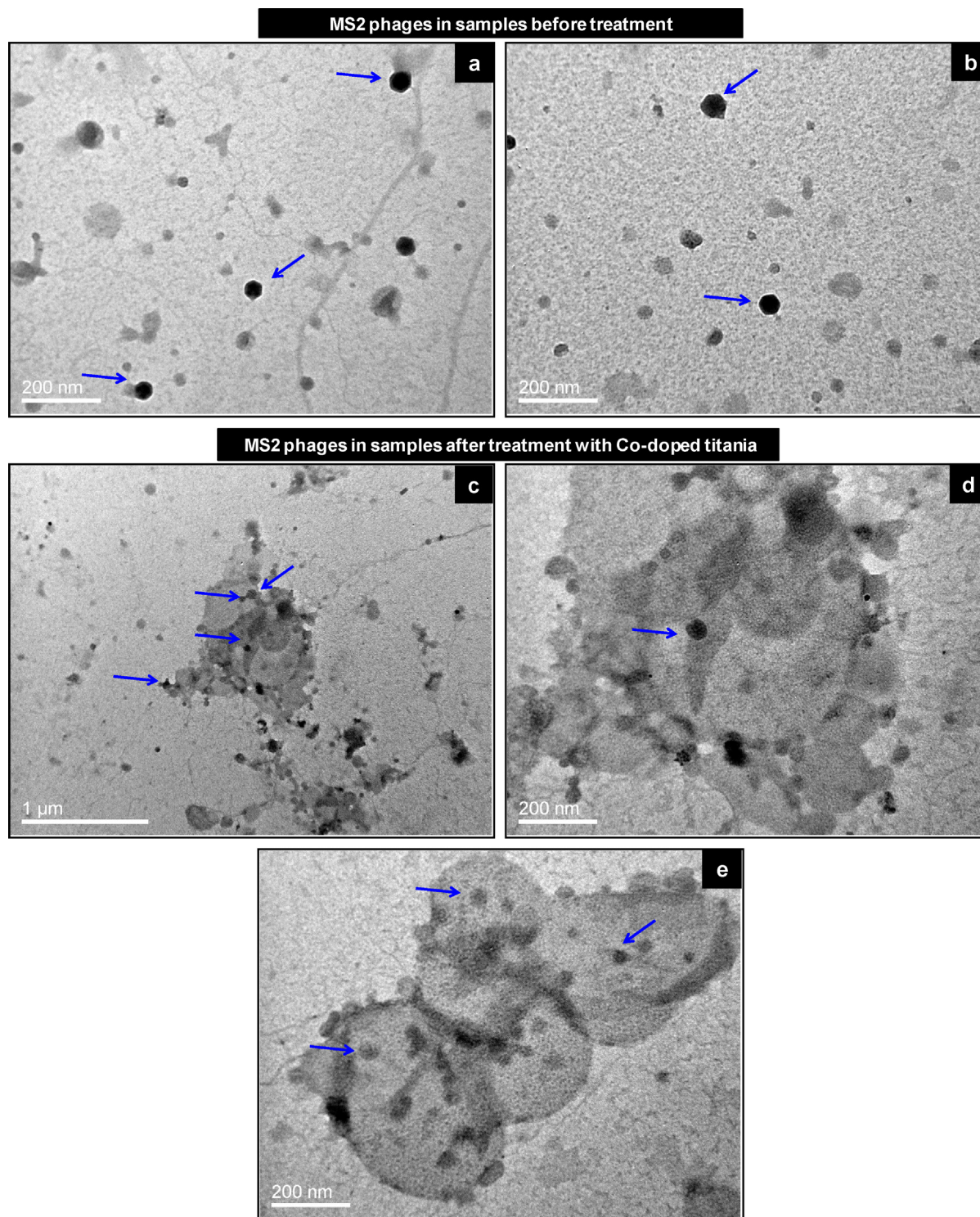
MS2 phages in samples before treatment

MS2 phages in samples after treatment with Co-doped titania

Fig. 4. TEM images of MS2 phages without treatment (a and b—negative controls) and after photocatalytic treatment in the presence of 0.3% Co:TiO$_2$ (c and d) and 1% Co:TiO$_2$ (e). Arrows point out MS2 phages in the samples. MS2 initial concentration in each experiment is 10^5 PFU/mL.

required than those recorded under simulated solar irradiation. In almost 60 min phage population reduction reached values of almost 3 and 2.5 Logs in the presence of 0.3 wt% Mn–TiO$_2$ and 0.3 wt% Co–TiO$_2$, respectively. Superior photocatalytic activity, in terms of phage inactivation, was observed again with the binary doped catalyst (0.1 wt% Mn/Co–TiO$_2$) as there was over 99% phage reduction within 45 min of treatment. The obtained inactivation curves under both simulated and natural solar light exhibited a tailing-off

period, which was more pronounced under the former conditions. This result is in consistence with another study, in which inactivation behavior of various phages was examined using UV and solar photocatalysis [38].

Retardation of the process may also be observed through the kinetic rate constants of inactivation curves. Disinfection rates could be fitted satisfactorily to a pseudo-first order kinetic expression and the computed kinetic rate constants are
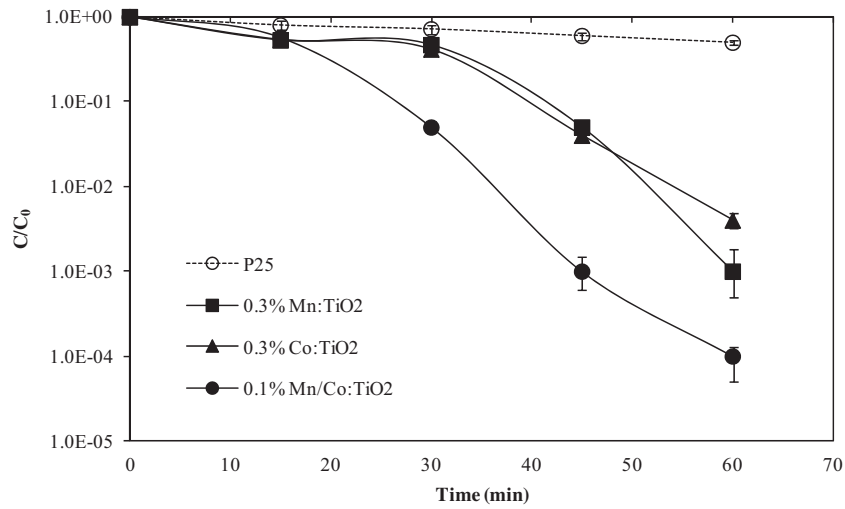
Fig. 5. MS2 phages inactivation in sewage samples under natural sunlight and in the presence of different doped TiO₂ catalysts. Catalyst concentration is 100 mg/L and MS2 initial concentration is 10^5 PFU/mL.
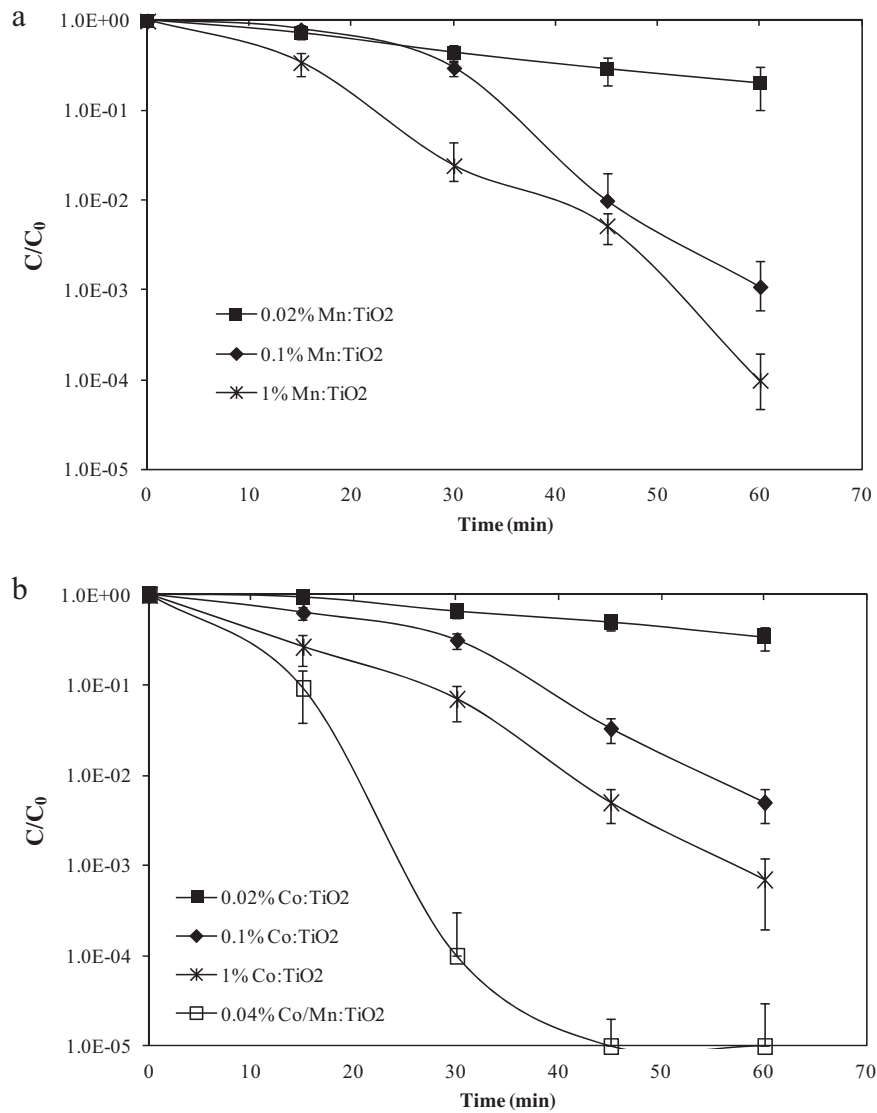

Fig. 6. Effect of wavelength on the inactivation of MS2 phages with the use of a 420 nm cutoff filter in the presence of different (a) Mn-doped and (b) Co-doped and binary Mn/Co doped TiO₂ catalysts under simulated solar irradiation. Catalyst concentration is 100 mg/L and MS2 initial concentration is 10^5 PFU/mL.
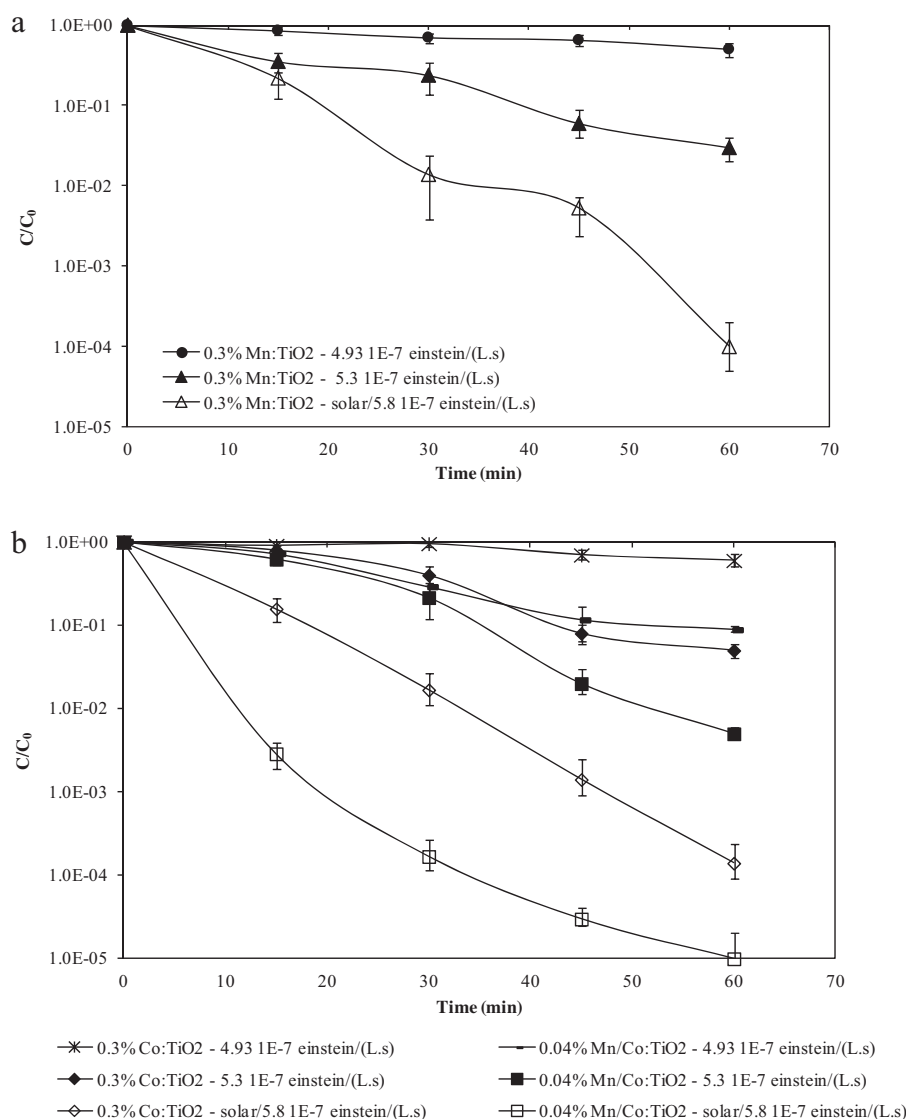
Fig. 7. Effect of photon flux on the inactivation of MS2 phages with the use of different (a) Mn-doped and (b) Co-doped and binary Mn/Co doped TiO_2 catalysts. Catalyst concentration is 100 mg/L and MS2 initial concentration is 10^5 PFU/mL.

Table 1
Rate constants and linear fit (R^2) of phage inactivation kinetics.

Experimental conditions−Catalyst		k (min^{-1})	R^2
Simulated solar irradiation	0.3% Mn:TiO$_2$	0.053	0.91
	0.3% Co:TiO$_2$	0.11	0.96
	0.1% Mn/Co:TiO$_2$	0.221	0.98
Natural sunlight	0.3% Mn:TiO$_2$	0.032	0.94
	0.3% Co:TiO$_2$	0.04	0.93
	0.1% Mn/Co:TiO$_2$	0.057	0.96

presented in Table 1. Under the operational conditions of the current study phage decay was delayed by a factor within the range of 1.6–3.8 when disinfection was performed outdoors. Comparing rate constants [k (min^{-1})] higher values were computed for experiments under simulated solar irradiation than those under natural sunlight. For example, using the catalyst with the binary dopant (0.1 wt% Mn/Co–TiO$_2$), a 4 Log reduction was recorded in almost 35 min under simulated solar irradiation, while the same decay was achieved in over 50 min with natural sunlight. Similar retardation periods were shown by Suri et al., who studied inactivation rates of *E. coli* in water with Pt- and Ag-doped titania. Although sunlight was effective for activating the photocatalysts and eliminating the

bacterial indicator, the decay rates were slightly slower compared to those obtained with artificial light [27]. Conversely, in another work with the same metal dopants and *Bacillus stearothermophilus* endospores as the microbial target, natural sunlight proved to be superior to artificial light. However, it should be noted that the solar irradiation alone, without the addition of any catalyst, reduced the amount of colony forming endospores causing detrimental damages to the cells [13]. In the case of MS2 phages, the resistant nature of the virus requires significant high periods, which in certain cases may reach 24 h in order to observe a 4 Log decay with sunlight exposure [6].

Apart from the light intensity dependence, there are other factors that may differentiate the course of the process, of which the composition is enlisted among the most determinants. Photocatalytic activity may be outweighed by other effects, reducing or varying the observed microbial inactivation rates. In our case experimental matrix was wastewater, whose chemical parameters concentration did not vary significantly among the samples tested in each case. Nevertheless, it is considered as a "complex" sample that affects photocatalytic process to a considerable extent. Particulate matter, various organic and inorganic components contained in wastewater aid in the resistance of microorganisms to disinfection,

as they physically shield microorganisms interfering in the whole process. Moreover, they inhibit light penetration, which is required in order to produce reaction throughout the bulk of the liquid. Inhibition of photocatalytic killing of bacteriophage MS2 may occur if there is phosphate in the suspension. It is assumed that phosphate ions block the binding of the phage to the TiO$_2$ particles. The same action has been recorded when disinfection is referred to bacterial populations [21]. However, based on our results and in accordance to other studies, photocatalysis proved to be effective method for wastewater disinfection in artificial and natural conditions [6,27]. Additional asset of this technique is the fact that it may contribute in reducing the precursors responsible for the formation of disinfection by-products (DBPs), which are often produced, especially if wastewater disinfection practice involves chlorination [27].

3.2.3. Effect of wavelength and photon flux on the inactivation of MS2 phages

As has already been presented in the current study, Mn-, Co- and Mn/Co doping red-shifted catalysts absorption to the visible region up to 600 nm and decreased significantly their band gaps, compared to the commercial P25. Outcomes of our photocatalytic experiments highlight the efficacy of metal-doped catalysts in terms of MS2 phage inactivation in wastewater samples under simulated and natural solar irradiation. In order to further support our findings regarding the visible-light activity of the prepared catalysts, a set of experiments was performed using a filter to cut-off UV light below 420 nm and the results are depicted in Fig. 6. Exclusion of UV light does not seem to deteriorate disinfection efficiency, as all tested metal-doped catalysts achieved satisfactory decay of MS2 population. Population reduction was recorded in the ranges of 75–99% and 65–99% when Mn- and Co- doped catalysts were used, respectively. Interestingly, when catalysts with the lowest dopant concentration were applied (i.e. 0.02 wt% Mn:TiO$_2$ and 0.02 wt% Co:TiO$_2$) inactivation curves exhibited clearly the well known "shoulder" region, covering the first period of the reaction. The initial delay is associated with the induction period, which is before the level of radicals produced becomes harmful to the microorganisms [21]. Even with this small retardation period phages decreased by 75% and 65% in the presence of the Mn- and Co-dopant, respectively. Higher inactivation rate was recorded in the presence of the binary dopant catalyst, with which 99.9% reduction of MS2 was achieved within 20 min of treatment, while shorter period was required when the filter was not employed (Fig. 2). Noticeably, no photocatalytic activity above 420 nm was observed with the commercial P25 throughout the process (data not shown). On the whole, efficiency of the metal-doped catalysts was improved with the addition of UV light, but this finding should be evaluated in relation to the organism in question. MS2 phage lacks any cellular structure, being therefore rather susceptible to UV irradiation and the induced nucleic acid disruption [38].

The contribution of UV light to the overall photocatalytic activity is conspicuous in Fig. 7 as some experiments with 0.3 wt% Mn-, 0.3 wt% Co- and 0.04 wt% Mn/Co co-doped TiO$_2$ were also performed at reduced photon fluxes. As it may be seen for example, 1 Log removal of phages occurred at 5.8×10^{-7} E/(L s) with 0.3 wt% Co:TiO$_2$ in 20 min of treatment, while three-fold period was required to achieve the same inactivation at 5.3×10^{-7} E/(L s). The same trend followed inactivation with the binary dopant catalyst. Reducing photon flux at 4.93×10^{-7} E/(L s) the density of the residual phages in the reaction solution was high. In the case of 0.3 wt% Mn:TiO$_2$ only 26.5% of phage population was eliminated. These findings corroborate the beneficial role of dopants, which show positive results particularly for visible light activity in TiO$_2$. Once metal-dopants are incorporated into the TiO$_2$ framework and narrow band gaps are achieved, the spectral response of the catalyst extends well into the visible region and enhanced activity may be

recorded with respect to water/wastewater disinfection, as it was revealed in this work. Therefore, treatment mediated by natural solar energy, which is an economically viable process, can be used successfully for the removal of various and diverse microorganisms, included in the aquatic environment.

4. Conclusions

Inactivation rates of MS2 bacteriophage driven by solar radiation with the use of metal-doped catalysts were investigated. Taking into account all benefits obtained by metal incorporation onto TiO$_2$ surface, manganese- and cobalt-doped titania nanoparticles were introduced in sewage samples with the view to study phage behavior during photocatalytic treatment. The main conclusions extracted from this work are as follows:

- Metal doping TiO$_2$ led to considerable narrow band gaps and extended spectral response into the visible region.
- MS2 phage population reduction reached the level of 99.9% in sewage samples under simulated solar irradiation within 60 min in the presence of the prepared metal-doped catalysts, with the latter enhancing the overall process.
- The effect of different dopants concentration was apparent up to a certain limit. Highest doping levels did not necessarily resulted in complete or rapid inactivation of the phage.
- The process was retarded under natural solar light and longer periods were required for total phage removal from the reaction solution.
- Catalysts with the binary dopant exhibited the best photocatalytic activity in all cases, highlighting the fact that composite dopants induce a synergistic effect.
- All tested catalysts were considerably sensitized in the absence of UV light. The visible light activity of catalysts and the recombination delay of the electron–hole pair account for the improved properties of metal-doped titania.

References

[1] R.M. Maier, I.L. Pepper, C.P. Gerba, Environmental Microbiology, second ed., Academic Press, Elsevier, San Diego, California, USA, 2009.
[2] S. Jebri, F. Hmaied, J. Jofre, M. Yahya, J. Mendez, I. Barkallah, M. Hamdi, Water Res. 47 (2013) 3673–3678.
[3] F. Lucena, A.E. Duran, A. Morón, E. Calderón, C. Campos, C. Gantzer, S. Skraber, J. Appl. Microbiol. 97 (2004) 1069–1076.
[4] J.Y. Kim, C. Lee, D.L. Sedlak, J. Yoon, K.L. Nelson, Water Res. 44 (2010) 2647–2653.
[5] B.M. Pecson, L.V. Martin, T. Kohn, Appl. Environ. Microbiol. 75 (2009) 5544–5554.
[6] B.M. Pecson, L. Decrey, T. Kohn, Water Res. 46 (2012) 1763–1770.
[7] K.J. Charles, F.C. Souter, D.L. Baker, C.M. Davies, J.F. Schijven, D.J. Roser, D.A. Deere, P.K. Priscott, N.J. Ashbolt, Water Res. 42 (2008) 3047–3056.
[8] S. Malato, P. Fernández-Ibáñez, M.I. Maldonado, J. Blanco, W. Gernjak, Catal. Today 147 (2009) 1–59.
[9] J. Koivunen, H. Heinonen-Tanski, Water Res. 39 (2005) 1519–1526.
[10] L. Hu, M.A. Page, T. Sigstam, T. Kohn, B.J. Mariñez, T.J. Strathmann, Environ. Sci. Technol. 46 (2012) 12079–12087.
[11] D. Gerrity, H. Ryu, J. Crittenden, M. Abbaszadegan, J. Environ. Sci. Health, A: Environ. Sci. Eng. 43 (2008) 1261–1270.
[12] M. Vijay, K. Ramachandran, P.V. Ananthapadmanabhan, B. Nalini, B.C. Pillai, F. Bondioli, A. Manivannan, R.T. Narendhirakannan, Curr. Appl. Phys. 13 (2013) 510–516.

[13] C. Berberidou, I. Paspaltsis, E. Pavlidou, T. Sklaviadis, I. Poulios, Appl. Catal., B: Environ. 125 (2012) 375–382.
[14] T. Sato, M. Taya, Biochem. Eng. J. 28 (2006) 303–308.
[15] H. Ishiguro, Y. Yao, R. Nakano, M. Hara, K. Sunada, K. Hashimoto, J. Kajioka, A. Fujishima, Y. Kubota, Appl. Catal., B: Environ. 129 (2013) 56–61.
[16] M. Cho, H. Chung, W. Choi, J. Yoon, Appl. Environ. Microbiol. 71 (2005) 270–275.
[17] M. Cho, E.L. Cates, J.H. Kim, Water Res. 45 (2011) 2104–2110.
[18] T.M. Tsai, H.H. Chang, K.C. Chang, Y.L. Liu, C.C. Tseng, J. Chem. Technol. Biotechnol. 85 (2010) 1642–1653.
[19] B. Wang, M.K.H. Leung, X.Y. Lu, S.Y. Chen, Appl. Energy 112 (2013) 1190–1197.
[20] M. Pelaez, N.T. Nolan, S.C. Pillai, M.K. Seery, P. Falaras, A.G. Kontos, P.S.M. Dunlop, J.W.J. Hamilton, J.A. Byrne, K. O'Shea, M.H. Entezari, D.D. Dionysiou, Appl. Catal., B: Environ. 125 (2012) 331–349.
[21] I.B. Ditta, A. Steele, C. Liptrot, J. Tobin, H. Tyler, H.M. Yates, D.W. Sheel, H.A. Foster, Appl. Microbiol. Biotechnol. 79 (2008) 27–133.
[22] J.G. McEvoy, W. Cui, Z. Zhang, Catal. Today 207 (2013) 191–199.
[23] H.U. Lee, S.C. Lee, S. Choi, B. Son, S.M. Lee, H.J. Kim, J. Lee, Chem. Eng. J. 228 (2013) 756–764.
[24] H. Feng, M.H. Zhang, L.E. Yu, Appl. Catal., A: Gen. 413–414 (2012) 238–244.
[25] V.D. Binas, K. Sambani, T. Maggos, A. Katsanaki, G. Kiriakidis, Appl. Catal., B: Environ. 113–114 (2012) 79–86.
[26] M.B. Fisher, D.A. Keane, P. Fernández-Ibáñez, J. Colreavy, S.J. Hinder, K.G. McGuigan, S.C. Pillai, Appl. Catal., B: Environ. 130–131 (2013) 8–13.
[27] R.P.S. Suri, H.M. Thornton, M. Muruganandham, Environ. Technol. 33 (2012) 1651–1659.
[28] S. Swetha, S.M. Santhosh, R.G. Balakrishna, Photochem. Photobiol. 86 (2010) 1127–1134.
[29] C. Karunakaran, A. Vijayabalan, G. Manikandan, P. Gomathisankar, Catal. Commun. 12 (2011) 826–829.
[30] D. Venieri, A. Fraggedaki, M. Kostadima, E. Chatzisymeon, V. Binas, A. Zachopoulos, G. Kiriakidis, D. Mantzavinos, Appl. Catal., B: Environ. 154–155 (2014) 93–101.
[31] K.L. Willett, R.A. Hites, J. Chem. Educ. 77 (2000) 900–902.
[32] E.S. Galbavy, K. Ram, C. Cort Anastasio, J. Photochem. Photobiol., A: Chem. 209 (2010) 186–192.
[33] L. Rizzo, D. Sannino, V. Vaiano, O. Sacco, A. Scarpa, D. Pietrogiacomi, Appl. Catal., B: Environ. 144 (2014) 369–378.
[34] L. Rizzo, A. Della Sala, A. Fiorentino, G. Li Puma, Water Res. 53 (2014) 145–152.
[35] K. Song, J. Zhou, J. Bao, Y. Feng, J. Am. Ceram. Soc. 91 (2008) 1369–1371.
[36] V.C. Papadimitriou, V.G. Stefanopoulos, M.N. Romanias, P. Papagiannakopoulos, K. Sambani, V. Tudose, G. Kiriakidis, Thin Solid Films 520 (2011) 1195–1201.
[37] J.C. Sjogren, R.A. Sierka, Appl. Environ. Microbiol. 60 (1994) 344–347.
[38] D. Misstear, L.W. Gill, J. Photochem. Photobiol., B: Biol. 107 (2012) 1–8.
[39] L.W. Sinton, C.H. Hall, P.A. Lynch, R.J. Davies-Colley, Appl. Environ. Microbiol. 68 (2002) 1122–1131.